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Phase II Prostate Cancer Vaccine Trial: Report of a Study Involving 37 Patients With Disease Recurrence Following Primary Treatment

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BACKGROUND. A phase II trial was conducted to assess the efficacy of infusions of dendritic cells (DC) and two HLA-A2-specific prostate-specific membrane antigen (PSMA) peptides (PSM-P1 and -P2). This report describes the evaluation of 37 subjects admitted with presumed local recurrence of prostate cancer after primary treatment failure.

METHODS. All subjects received six infusions of DC pulsed with PSM-P1 and -P2 at 6-week intervals. Clinical monitoring was conducted pre-, during, and post-phase II study. Data included: complete blood count, bone and total alkaline phosphatase, prostate markers, physical examination, performance status, bone scan, ProstaScint® scan, and chest X-ray, as well as other assays to monitor cellular and humoral immune responses.

RESULTS. One complete and 10 partial responders were identified from this group based on National Prostate Cancer Project criteria, or on a 50% reduction of prostate-specific antigen (PSA), or on a significant resolution in lesions (biopsy-proven when possible) on ProstaScint® scan.

CONCLUSIONS. About 30% of study participants in this group showed a positive response at the conclusion of the trial. This study suggests that DC-based cancer vaccines may provide an alternative therapy for prostate cancer patients whose primary treatment failed. *Prostate 39:54–59, 1999.* © 1999 Wiley-Liss, Inc.

KEY WORDS: **dendritic cells; prostate-specific membrane antigen; immunotherapy; cancer vaccine, local recurrence; prostate cancer; clinical trial**

INTRODUCTION

Current treatments for early-stage, localized prostate cancers are radical prostatectomy and radiation therapy. These procedures may exhibit failure rates of more than 20% [1]. As a result, there has been an increasing number of treated patients who either manifest metastatic disease or who are at very high risk for the development of such a state. The options for these primary treatment failures, which include hormonal, chemotherapeutic, and radiation strategies, can be limited in terms of duration and efficacy [2]. Immunotherapeutic approaches to cancer treatment have shown some promise in experimental studies [3–5].

One of the more recent advances in cancer immunotherapy utilizes autologous antigen-presenting cells

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(APC) and cancer antigens as cancer vaccines [6]. APC express factors which are necessary in efficient presentation of antigens for an effective T-cell activation. Dendritic cells (DC) are considered the most potent APC of the immune system [7]. Our approach to a prostate cancer vaccine utilizes cultured patient DC and prostate-specific membrane antigen (PSMA) peptides to activate prostate cancer-specific autologous T cells in vivo [8]. Our initial phase I trial demonstrated that infusions of autologous DC and HLA-A2-specific PSMA peptides were well-tolerated by 51 study participants with hormone-refractory advanced prostate cancer. In addition, favorable cellular immune responses were observed in 7 partial responders, identified based on National Prostate Cancer Project (NPCP) criteria, or on a 50% reduction of prostate-specific antigen (PSA) level [9,10].

Our phase II clinical trial enrolled a total of 107 subjects who received six infusions of DC pulsed with two HLA-A2-specific PSMA peptides. Sixty-six subjects were admitted with hormone-refractory metastatic prostate cancer. We previously reported that from this group, 17 responders (29.3%) were identified from a total of 58 evaluable subjects based on NPCP criteria, or >50% reduction of PSA level, or significant improvement in ProstaScint® scan [11,12]. This report describes the evaluation of 37 patients admitted with recurrence of prostate cancer after primary treatment.

MATERIALS AND METHODS

Reagents and Cytokines

PSMA peptides with HLA-A0201-specific motif (PSM-P1, LLHETDSAV; PSM-P2, ALFDIESKV) were synthesized and purified (95% purity) by Peninsula Laboratories, Inc. (Belmont, CA) and obtained as a lyophilized powder. The powder was dissolved in 0.9% saline (USP 0.9%, sodium chloride injection, American Reagent Laboratories, Shirley, NY) to a concentration of 2 mg/ml. The peptide solution was sterilized using 0.2-μm filtration. Granulocyte-macrophage colony-stimulating factor (GM-CSF), approved for human use, was provided by Immunex Corp. (Seattle, WA). Interleukin-4 (IL-4) was purchased from Peprotech, Inc. (Rocky Hill, NJ).

DC Culture

Each participant was subjected to leukapheresis at the Fred Hutchinson Cancer Research Center (Seattle, WA) (or at Northwest Hospital by alternative blood draws) prior to the start of the trial. DC were cultured as described previously [9,11]. In short, peripheral blood mononuclear cells (PBMC) were isolated using Histopaque 1077 Ficoll (Sigma Chemical Co., St. Louis, MO) density gradient. PBMC were resuspended in complete medium (OPTIMEM medium, from GIBCO-BRL, Grand Island, NY, and 5% heat-inactivated autologous plasma) and plated in a 75-cm^2 tissue culture flask (2–3 × 10^7 cells/flask). Cell suspensions were incubated in a humidified incubator (37°C, 5% CO_2) for 60 min. Nonadherent cells were removed, and adherent cells were washed gently with warm (37°C) complete medium. DC propagation medium (DCPM: complete medium, 500 units/ml GM-CSF and 500 units/ml IL-4) was added to the adherent cells (10 ml/flask). These cells were cultured for 7 days.

Administration of DC Pulsed With PSMA Peptides

Cultured DC were incubated for 2 hr in the presence of 10 μg/ml PSM-P1 and -P2 peptides, washed, resuspended in 10 ml injection grade saline, and delivered to the Northwest Hospital Day Surgery/Short Stay Unit. The DC suspension was infused over 30 min with a total volume of 100 ml 0.9% saline. Six infusions of autologous DC-pulsed PSM-P1 and -P2 peptides were administered at 6-week intervals. All study participants were subjected to clinical monitoring, as described below.

Clinical Monitoring

Patients were followed before, during, and after treatment with periodic PSA (Tandem-E PSA kit, Hybritech Incorporated, San Diego, CA), free PSA (Tandem-R PSA kit, Hybritech Incorporated), PSMA (in-house Western blot assay), complete blood counts, CHEM-22, bone alkaline phosphatase (Tandem-R Ostase kit, Hybritech Incorporated), inital chest X-ray, bone scans, and ProstaScint® scans [13,14]. All testing was conducted on an outpatient basis at Northwest Hospital. A delayed-type hypersensitivity (DTH) test to measure patients' general immune response activity was conducted at the beginning of this trial and repeated after conclusion of the study. Recall antigens tested were: streptococcus, tuberculin, glycerin, candida, trychophyton, and proteus. Patients were also evaluated every infusion cycle by one of the study physicians.

RESULTS

Study Population

Forty-one patients admitted with locally advanced prostate cancer were initially enrolled in this clinical

TABLE I. Study Population Summary (n = 37)*

Age		
Mean	68	
Median	70	
Range	51–80	
HLA-A2		
Positive	28	
Negative	9	
Prostate cancer stage		
$C(T_3)$	4/37 (11%)	
$D_0(T_4N_0M_0)$	17/37 (46%)	
$D_1(T_4N_{1-3}M_0)$	4/37 (11%)	
$D_2(T_4N_{1-3}M_{1a-c})$	12/37 (32%)	
Primary prostate cancer therapy		
Radical prostatectomy		21 (57%)
Radiation therapy		9 (24%)
Brachytherapy		4 (11%)
Hormone therapy		3 (8%)

*Out of 41 subjects who were initially enrolled in the study, 37 participants received at least one infusion.

trial. Four subjects were unable to participate and never received a single infusion, leaving 37 subjects who were evaluable. All participants were subjected to prestudy tests, which included prostate markers, chest X-ray, bone-scan, and ProstaScint® scan. These tests allowed for determination of their clinical staging at the start of the study. The clinical staging, HLA-A2 status, and primary prostate cancer therapy history are summarized in Table I. The most common primary types of treatments given to this study population were: radical prostatectomy (21/37; 57%), radiation therapy (9/37; 24%), and brachytherapy (4/37; 11%). In addition, 13/37 (35%) subjects underwent a secondary line of treatments, which included radiation and brachytherapy. Three patients had hormone therapy as the initial and only treatment. One on luteinizing hormone-releasing hormone (LHRH) agonist only was a partial responder (Table II). One subject was on antiandrogen only, and one was postorchiectomy. These 2 subjects did not show any significant change in clinical response status. The majority of this population (27/37; 73%) had a history of rising PSA prior to enrollment to this study (range, 0.4–33.9 ng/ml). The pretreatment PSA level of 23 subjects (62%) was below 4 ng/ml, while 7 subjects (19%) were between 4–10 ng/ml, and 7 subjects (19%) were above 10 ng/ml.

Six infusions of DC pulsed with PSM-P1 and -P2 peptides were administered at 6-week intervals. The numbers of DC infused (average, 18 million; range, 6–30 million) varied with each patient, based on the number of cells obtained from each culture. Thirty-seven study participants were evaluated for response, based on NPCP criteria, plus a 50% decrease in PSA, or significant improvements in repeat ProstaScint® scan. All 37 subjects completed the six scheduled infusions and follow-up evaluations. Ten subjects (27%) were identified as partial responders, and one (4%) as a complete responder. Among the nonresponders, 18 subjects (49%) exhibited disease progression, and 8 (22%) showed no significant change in disease state (Fig. 1).

In addition to clinical monitoring, each patient was also subjected to delayed-type hypersensitivity (DTH) skin testing to measure general immune response activity. These tests were conducted before and after the study. In the responder group, 6/11 (55%) subjects exhibited no change, 2/11 (18%) showed an increase, and 3/8 (27%) showed a decrease in skin reactivity. In the nonresponder group, 11/26 (42%) subjects exhibited no change, 4/26 (15%) subjects showed increased skin reactivity, and 10/26 (39%) showed decreased skin reactivity. One subject from the nonresponder group did not receive a repeat skin test. The average poststudy Karnofsky scores for the responder and nonresponder groups were both 100.

Profiles of Responders

Eleven subjects (30%) in this group showed significant improvements detected by the clinical evaluations conducted both during the study and after completion of all six infusions. These subjects were evaluated for an average of 341 days, which included six infusion cycles and a follow-up observation period. The profiles of these 11 responders, including their study entry disease stage, HLA-A2 status, and primary therapy, are summarized in Table II. Nine responders (82%) were HLA-A2-positive. One subject was identified as a complete responder (CR), and 10 others as partial responders (PR). Three of the partial responders showed at least 50% decline in serum PSA level, with maintenance of the decline on at least three separate determinations spaced at least 2 weeks apart (subjects 1, 4, and 5). Repeat ProstaScint® scans demonstrated one case of complete resolution (subject 9), and 9 cases of significant regression of cancer lesions of the lymph nodes and/or prostate bed. Prostate biopsy was performed to confirm ProstaScint® data involving prostate bed lesions (subjects 2, 3, 6, 7,and 11). One subject (subject 4) showed significant regression of clinically unsuspected metastatic lesions of the bone, as detected by pre- and poststudy bone scans.

Figure 2 depicts PSA levels of subject 4 throughout the study and observation period. This subject was a postradical prostatectomy with a PSA level of over 3 ng/ml at the start of the study and a normal ProstaScint® scan. His prestudy bone scan showed foci of intense uptakes, typical of metastatic disease in the left iliac crest, and in the right tenth rib. The decrease

TABLE II. Eleven Responders Identified From 37 Study Participants*

Subject	Stage	HLA-A2	Primary therapy	Average DC infused	Clinical response	Criteria for response[a]
1	D2	+	Hormone	19 million	PR	PSA, ProstaScint®
2	C	+	Brachytherapy	20 million	PR	ProstaScint®, negative biopsy[b]
3	D0	+	Prostatectomy	15 million	PR	ProstaScint®, negative biopsy[b]
4	D2	+	Prostatectomy	20 million	PR	PSA, bone scan
5	D2	+	Prostatectomy	16 million	PR	PSA, ProstaScint®
6	D1	−	Prostatectomy	22 million	PR	ProstaScint®, negative biopsy[b]
7	D0	−	Radiation	22 million	PR	ProstaScint®, negative biopsy[b]
8	D2	+	Prostatectomy	19 million	PR	ProstaScint®
9	D2	+	Prostatectomy	17 million	CR	ProstaScint®
10	D2	+	Radiation	16 million	PR	ProstaScint®
11	D0	+	Prostatectomy	15 million	PR	ProstaScint®, negative biopsy[b]

*CR, complete response; PR, partial response.
[a]Evaluation of clinical response was based on National Prostate Cancer Project criteria, plus more than 50% reduction in PSA, or significant improvement in repeat ProstaScint® scan.
[b]Biopsy was performed to confirm all ProstaScint® scan data involving the prostate bed area.

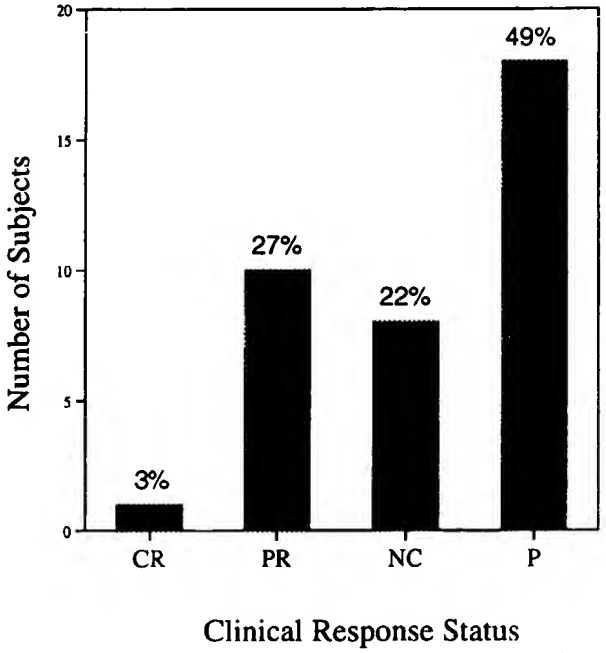

Fig. 1. Clinical evaluation of 37 subjects admitted with presumed local recurrence of prostate cancer after a primary treatment. The number and percentage of subjects in four clinical response categories to the therapy are shown. CR, complete response; PR, partial response; NC, no change; P, progression.

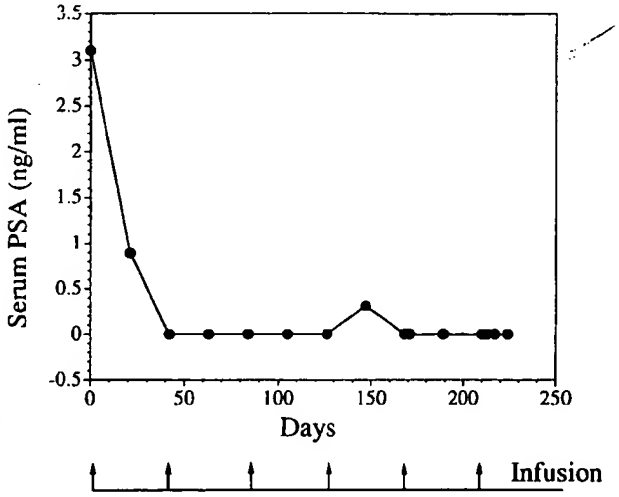

Fig. 2. Decrease of prostate-specific antigen levels in a partial responder. Serum PSA levels (depicted by solid circles) were measured on the day of each infusion and 3 weeks after that throughout the trial. More frequent testing was done during the poststudy evaluation period. Arrows indicate corresponding infusions.

of serum PSA level was detected after the first infusion, and reached a baseline level on the day of his second infusion. This baseline PSA level was maintained throughout the rest of the study and poststudy evaluation period (190 days). The repeat bone scan showed two marker lesions with decreased intensity.

Subject 8 had a radical prostatectomy as a primary treatment, followed by radiation therapy. Serum PSA stayed at baseline level throughout the study, and the bone scans were negative. The prestudy ProstaScint® scan showed metastatic lesions in the mesenteric and in the left supraclavicular lymph nodes, as well as local recurrence of prostate carcinoma. Comparison with a repeat ProstaScint® scan conducted at the conclusion of the study suggested disease regression in both lymph nodes and prostate fossa.

Subject 9 had a history of radical prostatectomy, and a negative bone scan. The entry ProstaScint® scan showed no pathologic uptake in the prostate fossa, but

moderately intense uptake in the periaortic and right common iliac lymph nodes. Poststudy ProstaScint® scan showed resolution of lesions in both lymph node areas. PSA stayed at a low baseline level throughout the study.

Subject 10 had radiation therapy as a first treatment. He subsequently underwent cryosurgery. PSA stayed at baseline level throughout the study, and the bone scans were negative. The entry ProstaScint® scan suggested the presence of metastatic lesions in the lower paraaortic and iliac regions. A repeat scan conducted after the sixth infusion showed no evidence of lymph node metastases, but an increase uptake in the prostate fossa. A biopsy was performed and the result was negative.

DISCUSSION

This report describes the clinical evaluation of a group of 37 subjects with a history of local recurrent prostate cancer, who participated in a phase II clinical trial involving six administrations of autologous DC pulsed with two HLA-A2-specific PSMA peptides. Thirty percent of the study population (11/37) showed significant clinical response to the therapy. The average total evaluation period was 341 days. Ten subjects were partial responders, and one subject was identified as a complete responder based on NPCP criteria, >50% reduction in PSA level, and/or significant improvement in repeat ProstaScint® scan. This study suggested that DC-based cancer vaccines may provide an alternative therapy for prostate cancer patients whose disease recurred after primary or secondary treatments.

Similar to our previous report on a group of patients with hormone-refractory metastatic prostate cancer who received the same treatment, comparisons of pre-and post-DTH skin testing using recall antigen suggested some correlation between the state of general immune response and clinical response [12]. Among the responder groups, a majority of the population (8/11; 73%) maintained or improved the state of their immune reactivity, while only 3/8 (27%) showed a decrease. In contrast, 10/26 (39%) nonresponders showed a reduction in skin test reactivity, while 15/26 (57%) maintained or improved their general immune reactivity. Other studies to monitor specific T-cell-mediated immune responses, including T-cell lymphoproliferative assays, interferon-γ, and interleukin-10 secretions upon presentation of PSMA peptides in vitro, and specific cytotoxic responses against a panel of prostate cancer lines, are still being conducted.

This phase II clinical trial evaluated the efficacy of administration of autologous DC pulsed with PSM-P1 and -P2 peptides to a total of 95 evaluable participants, which included subjects with hormone-refractory disease, as well as those admitted with a history of local recurrence following a primary treatment. Nearly 30% of the study participants (28/95) were identified as responders during an average total evaluation period of over 340 days [11,12]. This result showed promise for autologous DC-based therapy as an alternative strategy for advanced prostate cancer patients whose disease no longer responds to conventional primary or secondary treatments.

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